

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization  
International Bureau(43) International Publication Date  
15 August 2002 (15.08.2002)

PCT

(10) International Publication Number  
**WO 02/062387 A1**

- (51) International Patent Classification<sup>7</sup>: **A61K 45/00** [GB/GB]; GlaxoSmithKline, New Frontiers Science Park South, Third Avenue, Harlow, Essex CM19 5AW (GB).
- (21) International Application Number: PCT/GB02/00542
- (22) International Filing Date: 7 February 2002 (07.02.2002)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:  
0103031.1 7 February 2001 (07.02.2001) GB
- (71) Applicant (for all designated States except US):  
**SMITHKLINE BEECHAM P.L.C.** [GB/GB]; New Horizons Court, Brentford, Middlesex TW8 9EP (GB).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): **DOHERTY, Patrick** [GB/GB]; Molecular Neurobiology Group, MRC Centre for Developmental Biology, King's College London, Guy's Campus, London SE1 1UL (GB). **EICKHOLT, Britta, Johanna** [DE/GB]; Molecular Neurobiology Group, MRC Centre for Developmental Biology, King's College London, Guy's Campus, London SE1 1UL (GB). **SKAPER, Stephen, Drake** [US/GB]; GlaxoSmithKline, New Frontiers Science Park South, Third Avenue, Harlow, Essex CM19 5AW (GB). **WALSH, Frank, Sinclair**
- (81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.
- (84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).
- Published:**  
— with international search report
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: TREATMENT OF CONDITIONS WITH A NEED OF GSK-3 INHIBITION

(57) Abstract: A method of treatment for the promotion of nerve regeneration, including axonal regrowth, axonal outgrowth, and prevention of growth cone collapse, in cases of acute neuronal injury, such as crush injury, acute stroke, ischaemia, neurotraumatic insult, spinal cord injury and neurotrauma in humans or non-human mammals, which method comprises the administration of an effective, non-toxic and pharmaceutically acceptable amount of a GSK-3 inhibitor or a pharmaceutically acceptable derivative thereof.



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## TREATMENT OF CONDITIONS WITH A NEED OF GSK-3 INHIBITION

This invention relates to a novel treatment and in particular to a method of treatment for the promotion of nerve regeneration.

5 GSK-3 is a serine/threonine protein kinase composed of two isoforms ( $\alpha$  and  $\beta$ ) which are encoded by distinct genes. GSK-3 is one of several protein kinases which phosphorylates glycogen synthase (GS) (Embi *et al* Eur. J. Biochem. (107) 519-527 (1980)). The  $\alpha$  and  $\beta$  isoforms have a monomeric structure and are both found in mammalian cells. Both isoforms phosphorylate muscle glycogen synthase (Cross *et al* 10 Biochemical Journal (303) 21-26 (1994)) and these two isoforms show good homology between species (e.g. human and rabbit GSK-3 $\alpha$  are 96% identical).

Type II diabetes (or Non-Insulin Dependent Diabetes Mellitus, NIDDM) is a multifactorial disease. Hyperglycaemia is due to insulin resistance in the liver, muscle and other tissues coupled with inadequate or defective secretion of insulin from 15 pancreatic islets. Skeletal muscle is the major site for insulin-stimulated glucose uptake and in this tissue, glucose removed from the circulation is either metabolised through glycolysis and the TCA cycle, or stored as glycogen. Muscle glycogen deposition plays the more important role in glucose homeostasis and Type II diabetic subjects have defective muscle glycogen storage.

20 The stimulation of glycogen synthesis by insulin in skeletal muscle results from the dephosphorylation and activation of glycogen synthase (Villar-Palasi C. and Larner J. Biochim. Biophys. Acta (39) 171-173 (1960), Parker P J *et al.*, Eur. J. Biochem. (130) 227-234 (1983), and Cohen P. Biochem. Soc. Trans. (21) 555-567 (1993)). The phosphorylation and dephosphorylation of GS are mediated by specific kinases and 25 phosphatases. GSK-3 is responsible for phosphorylation and deactivation of GS, while glycogen bound protein phosphatase 1 (PP1G) dephosphorylates and activates GS. Insulin both inactivates GSK-3 and activates PP1G (Srivastava A K and Pandey S K Mol. and Cellular Biochem. (182) 135-141 (1998)).

30 Chen *et al* Diabetes (43) 1234-1241 (1994) found that there was no difference in the mRNA abundance of PP1G between patients with Type II diabetes and control patients, suggesting that an increase in GSK-3 activity might be important in Type II diabetes. It has also recently been demonstrated that GSK-3 is overexpressed in Type II diabetic muscle and that an inverse correlation exists between skeletal muscle GSK-3 $\alpha$  activity and insulin action (Nikoulina *et al* Diabetes 2000, 49 263-271). Overexpression 35 of GSK-3 $\beta$  and constitutively active GSK-3 $\beta$  (S9A, S9E) mutants in HEK-293 cells resulted in suppression of glycogen synthase activity (Eldar-Finkelman *et al.*, PNAS (93) 10228-10233 (1996)) and overexpression of GSK-3 $\beta$  in CHO cells, expressing both insulin receptor and insulin receptor substrate 1 (IRS-1), resulted in an impairment of insulin action (Eldar-Finkelman and Krebs PNAS (94) 9660-9664 (1997)). Recent 40 evidence for the involvement of elevated GSK-3 activity and the development of insulin resistance and type II diabetes in adipose tissue has emerged from studies undertaken in diabetes and obesity prone C57BL/6J mice (Eldar-Finkelman *et al.*, Diabetes (48) 1662-1666 (1999)).

GSK-3 has been shown to phosphorylate other proteins *in vitro* including the eukaryotic initiation factor eIF-2B at Serine<sup>540</sup> (Welsh *et al.*, FEBS Letts (421) 125-130 (1998)). This phosphorylation results in an inhibition of eIF-2B activity and leads to a reduction in this key regulatory step of translation. In disease states, such as diabetes, where there is elevated GSK-3 activity this could result in a reduction of translation and potentially contribute to the pathology of the disease.

Several aspects of GSK-3 functions and regulation in addition to modulation of glycogen synthase activity indicate that inhibitors of this enzyme may be effective in treatment of disorders of the central nervous system. GSK-3 activity is subject to inhibitory phosphorylation by PI 3 kinase-mediated or Wnt-1 class-mediated signals that can be mimicked by treatment with lithium, a low mM inhibitor of GSK-3 (Stambolic V., Ruel L. and Woodgett J.R. Curr. Biol. 1996 6(12): 1664-8).

GSK-3 inhibitors may be of value as neuroprotectants in treatment of acute stroke and other neurotraumatic injuries. Roles for PI 3-kinase signalling through PKB/akt to promote neuronal cell survival are well established, and GSK-3 is one of a number of PKB/akt substrates to be identified that can contribute to the inhibition of apoptosis via this pathway (Pap & Cooper, (1998) J. Biol. Chem. 273: 19929-19932). Evidence suggests that astrocytic glycogen can provide an alternative energy source to facilitate neuronal survival under conditions of glucose deprivation (for example see Ransom, B.R. and Fern, R. (1997) Glia 21: 134-141 and references therein). Lithium is known to protect cerebellar granule neurons from death (D'Mello *et al.*, (1994) Exp. Cell Res. 211: 332-338 and Volonte *et al* (1994) Neurosci. Letts. 172: 6-10) and chronic lithium treatment has demonstrable efficacy in the middle cerebral artery occlusion model of stroke in rodents (Nonaka and Chuang, (1998) Neuroreport 9(9): 2081-2084). Wnt-induced axonal spreading and branching in neuronal culture models has been shown to correlate with GSK-3 inhibition (Lucas & Salinas, (1997) Dev. Biol. 192: 31-44) suggesting additional value of GSK-3 inhibitors in promoting neuronal regeneration following neurotraumatic insult.

Tau and  $\beta$ -catenin, two known *in vivo* substrates of GSK-3, are of direct relevance in consideration of further aspects of the value of GSK-3 inhibitors in relation to treatment of chronic neurodegenerative conditions. Tau hyperphosphorylation is an early event in neurodegenerative conditions such as Alzheimer's disease (AD), and is postulated to promote microtubule disassembly. Lithium has been reported to reduce the phosphorylation of tau, enhance the binding of tau to microtubules, and promote microtubule assembly through direct and reversible inhibition of glycogen synthase kinase-3 (Hong M., Chen D.C., Klein P.S. and Lee V.M. J.Biol. Chem. 1997 272(40) 25326-32).  $\beta$ -catenin is phosphorylated by GSK-3 as part of a tripartite complex with axin, resulting in  $\beta$ -catenin being targetted for degradation (Ikeda *et al.*, (1998) EMBO J. 17: 1371-1384). Inhibition of GSK-3 activity is a key mechanism by which cytosolic levels of catenin are stabilised and hence promote  $\beta$ -catenin-LEF-1/TCF transcriptional activity (Eastman, Grosschedl (1999) Curr. Opin. Cell Biol. 11: 233). Rapid onset AD mutations in presenilin-1 (PS-1) have been shown to decrease the cytosolic  $\beta$ -catenin pool in transgenic mice. Further evidence suggests that such a reduction in available  $\beta$ -

catenin may increase neuronal sensitivity to amyloid mediated death through inhibition of  $\beta$ -catenin-LEF-1/TCF transcriptional regulation of neuroprotective genes (Zhang *et al.*, (1998) *Nature* 395: 698-702). A likely mechanism is suggested by the finding that mutant PS-1 protein confers decreased inactivation of GSK-3 compared with normal PS-1 (Weihl, C.C., Ghadge, G.D., Kennedy, S.G., Hay, N., Miller, R.J. and Roos, R.P. (1999) *J. Neurosci.* 19: 5360-5369).

International Patent Application Publication Number WO 97/41854 (University of Pennsylvania) discloses that an effective drug for the treatment of manic depression is lithium, but that there are serious drawbacks associated with this treatment. Whilst the precise mechanism of action of this drug for treatment of manic depression remains to be fully defined, current models suggest that inhibition of GSK-3 is a relevant target that contributes to the modulation of AP-1 DNA binding activity observed with this compound (see Manji *et al.*, (1999) *J. Clin. Psychiatry* 60 (suppl 2): 27-39 for review).

GSK-3 inhibitors may also be of value in treatment of schizophrenia. Reduced levels of  $\beta$ -catenin have been reported in schizophrenic patients (Cotter D, Kerwin R, al-Sarraj S, Brion JP, Chadwich A, Lovestone S, Anderton B, and Everall I. 1998 *Neuroreport* 9:1379-1383 ) and defects in pre-pulse inhibition to startle response have been observed in schizophrenic patients (Swerdlow *et al* (1994) *Arch. Gen. Psychiat.* 51: 139-154). Mice lacking the adaptor protein dishevelled-1, an essential mediator of Wnt-induced inhibition of GSK-3, exhibit both a behavioural disorder and defects in pre-pulse inhibition to startle response (Lijam N, Paylor R, McDonald MP, Crawley JN, Deng CX, Herrup K, Stevens KE, Maccaferri G, McBain CJ, Sussman DJ, and Wynshaw-Boris A. (1997) *Cell* 90: 895-905). Together, these findings implicate deregulation of GSK-3 activity as contributing to schizophrenia. Hence, small molecule inhibitors of GSK-3 catalytic activity may be effective in treatment of this mood disorder.

The finding that transient  $\beta$ -catenin stabilisation may play a role in hair development (Gat *et al* *Cell* (95) 605-614(1998)) suggests that GSK-3 inhibitors could be used in the treatment of baldness.

Studies on fibroblasts from the GSK-3 $\beta$  knockout mouse (Hoefflich KP *et al.*, *Nature* 2000, 406, 86-90) support a role for this kinase in positively regulating the activity of NF $\kappa$ B. This transcription factor mediates cellular responses to a number of inflammatory stimuli. Therefore, pharmacologic inhibition of GSK-3 may be of use in treating inflammatory disorders through the negative regulation of NF $\kappa$ B activity.

Lucas *et al.* (*J. Cell. Science*, 111, 1351, (1998)) have examined the effect of lithium and the protein ligand WNT-7a on axonal spreading and growth cone area. It is concluded that the cytoskeletal protein, MAP-1B, is a target for GSK-3, and that inhibition of GSK-3 leads to a reduction in phosphorylated MAP-1B. Since MAP-1B is implicated to play an important role in the binding of microtubules, inhibition of GSK-3 and subsequent reduction of phospho-MAP-1B, is stated to lead to less stable microtubules, thereby allowing for axonal remodelling. The effect that phosphorylation of MAP-1B by GSK-3 has on it's function in cells has also been reported (Goold *et al.*, *J. Cell. Science*, 112, 3373, (1999)).

Sayas *et al.* (*J. Biol. Chem.*, **274**, 37046, (1999)) have reported that the bioactive phospholipid, lysophosphatidic acid (LPA) causes growth cone collapse and neurite retraction in a cell line with a neuronal-like phenotype (SY-SH5Y cells).

Semaphorin 3A ("Sema 3A"), a repulsive axon guidance molecule, contributes to the correct wiring of the nervous system by channelling the growth of axons away from inappropriate territories (Nakamura *et al.*, *J. Neurobiol.*, 2000, **44**, 219). Following injury, the same molecule has been postulated to inhibit nerve regeneration *via* growth cone collapse (Pasterkamp *et al.*, *Mol. Cell. Neurosci.*, 1999, **13**, 143).

It has now surprisingly been indicated that inhibition of GSK-3 prevents the growth cone collapse response induced by Semaphorin 3A and furthermore that inhibition of the kinase leads to stimulation of axonal regeneration into otherwise inhibitory Semaphorin 3A territory. Thus, GSK-3 inhibitors may be useful in the promotion of nerve regeneration, for example, axonal regrowth, axonal outgrowth, and the prevention of growth cone collapse in cases of acute neuronal injury, such as crush injury, acute stroke, ischaemia, neurotraumatic insult, spinal cord injury and neurotrauma and chronic neurodegenerative conditions such as Alzheimer's disease, Parkinson's disease, neurodegenerative diseases, Lou Gehrig's disease, and multiple sclerosis.

International Patent Applications Publication Numbers WO 00/21927, WO 00/38675, WO 01/09106 and WO 01/74771 (SmithKline Beecham PLC) disclose certain compounds useful as GSK-3 inhibitors.

Accordingly, the present invention provides a method of treatment for the promotion of nerve regeneration, including axonal regrowth, axonal outgrowth, and prevention of growth cone collapse, in cases of acute neuronal injury, such as crush injury, acute stroke, ischaemia, neurotraumatic insult, spinal cord injury and neurotrauma in humans or non-human mammals, which method comprises the administration of an effective, non-toxic and pharmaceutically acceptable amount of a GSK-3 inhibitor or a pharmaceutically acceptable derivative thereof.

Accordingly, the invention also provides a method of treatment for the promotion of nerve regeneration, for example axonal regrowth, axonal outgrowth, and the prevention of growth cone collapse, in cases of chronic neurodegenerative conditions such as Alzheimer's disease, Parkinson's disease, neurodegenerative diseases, Lou Gehrig's disease, and multiple sclerosis, in humans or non-human mammals, which method comprises the administration of an effective, non-toxic and pharmaceutically acceptable amount of a GSK-3 inhibitor or a pharmaceutically acceptable derivative thereof.

In a preferred aspect, the present invention provides a method of treatment for the promotion of nerve regeneration, for example axonal regrowth, axonal outgrowth, and the prevention of growth cone collapse, in cases of Parkinson's Disease, Lou Gehrig's disease and multiple sclerosis in humans or non-human mammals, which method comprises the administration of an effective, non-toxic and pharmaceutically acceptable amount of a GSK-3 inhibitor or a pharmaceutically acceptable derivative thereof.

In a preferred aspect, said GSK-3 inhibitor is a compound described in WO 00/21927, WO 00/38675, WO 01/09106 and WO 01/74771 or a pharmaceutically acceptable derivative thereof.

5 Suitably, the promotion of nerve regeneration according to the present invention is a consequence of the prevention of growth cone collapse response induced by Semaphorin 3A.

Suitably, the promotion of nerve regeneration according to the present invention is a consequence of stimulation of axonal regeneration into otherwise inhibitory Semaphorin 3A territory.

10 A suitable neurodegenerative condition for treatment in accordance with the present invention is Alzheimer's disease.

A suitable neurodegenerative condition for treatment in accordance with the present invention is Parkinson's disease.

15 A suitable condition for treatment according to the present invention is multiple sclerosis.

A suitable condition for treatment according to the present invention is Lou Gehrig's disease.

20 Suitable GSK-3 inhibitors include a compound of formula (IA), or a derivative thereof, wherein a compound of formula (IA) is defined as being a compound of formula (I) as defined in WO 00/21927.

The suitable and preferred compounds of formula (IA) are those compounds of formula (I) defined as suitable and preferred in WO 00/21927. Particularly preferred compounds of formula (IA) are disclosed on page 50, at Example 116 ("SB-331371") and at page 67, Example A558 ("SB-415286") of WO 00/21927.

25 Suitable GSK-3 inhibitors include a compound of formula (IB), or a derivative thereof, wherein a compound of formula (IB) is defined as being a "Compound of Group (I)" as defined in WO 00/38675.

The suitable and preferred compounds of formula (IB) are those "Compound of Group (I)" defined as suitable and preferred in WO 00/38675.

30 Suitable GSK-3 inhibitors include a compound of formula (IB'), or a derivative thereof, wherein a compound of formula (IB') is defined as being a "Compound of Group (II)" as defined in WO 00/38675.

The suitable and preferred compounds of formula (IB') are those "Compound of Group (II)" defined as suitable and preferred in WO 00/38675.

35 Suitable GSK-3 inhibitors include a compound of formula (IC), or a derivative thereof, wherein a compound of formula (IC) is defined as being a compound of formula (I) as defined in WO 01/09106.

The suitable and preferred compounds of formula (IC) are those compounds of formula (I) defined as suitable and preferred in WO 01/09106.

40 Suitable GSK-3 inhibitors include a compound of formula (ID), or a derivative thereof, wherein a compound of formula (ID) is defined as being a compound of formula (I) as defined in WO 01/74771.

The suitable and preferred compounds of formula (ID) are those compounds of formula (I) defined as suitable and preferred in WO 01/74771.

A further suitable GSK-3 inhibitor for use in the present invention is described in Coghlan et al., *Chemistry & Biology*, September 2000, 7, 793, at page 795 as "SB-216763".

Where a GSK-3 inhibitor contains a chiral carbon atom and hence exists in one or more stereoisomeric forms, or where one or more geometric isomers exist, it will be appreciated that the method of the present invention encompasses all of the said forms of the GSK-3 inhibitor whether as individual isomers or as mixtures of isomers, including racemates.

Certain of the compounds of formulae (IA), (IB), (IB'), (IC) and (ID) may contain chiral atoms and/or multiple bonds, and hence may exist in one or more stereoisomeric forms. The present invention encompasses all of the isomeric forms of the compounds of formulae (IA), (IB), (IB'), (IC) and (ID) whether as individual isomers or as mixtures of isomers, including geometric isomers and racemic modifications.

Suitable derivatives of a GSK-3 inhibitor are pharmaceutically acceptable derivatives, for example salts and solvates.

Suitable derivatives of any particular GSK-3 inhibitor include those disclosed in the above mentioned publications.

Suitable pharmaceutically acceptable salts include salts of salts derived from appropriate acids, such as acid addition salts, or bases.

Suitable pharmaceutically acceptable salts include metal salts, such as for example aluminium, alkali metal salts such as lithium, sodium or potassium, alkaline earth metal salts such as calcium or magnesium and ammonium or substituted ammonium salts, for example those with lower alkylamines such as triethylamine, hydroxy alkylamines such as 2-hydroxyethylamine, bis-(2-hydroxyethyl)-amine or tri-(2-hydroxyethyl)-amine, cycloalkylamines such as bicyclohexylamine, or with procaine, dibenzylpiperidine, N-benzyl-b-phenethylamine, dehydroabietylamine, N,N'-bisdehydroabietylamine, glucamine, N-methylglucamine or bases of the pyridine type such as pyridine, collidine, quinine or quinoline.

Suitable acid addition salts include pharmaceutically acceptable inorganic salts such as the sulphate, nitrate, phosphate, borate, hydrochloride and hydrobromide and pharmaceutically acceptable organic acid addition salts such as acetate, tartrate, maleate, citrate, succinate, benzoate, ascorbate, methane-sulphonate,  $\alpha$ -keto glutarate and  $\alpha$ -glycerophosphate, especially the maleate salt.

Suitable pharmaceutically acceptable salts of a compound of formula (IA) are as disclosed in WO 00/21927.

Suitable pharmaceutically acceptable salts of a compound of formula (IB) are as disclosed in WO 00/38675.

Suitable pharmaceutically acceptable salts of a compound of formula (IB') are as disclosed in WO 00/38675.

Suitable pharmaceutically acceptable salts of a compound of formula (IC) are as disclosed in WO 01/09106.

Suitable pharmaceutically acceptable salts of a compound of formula (ID) are as disclosed in WO 01/74771.

Suitable pharmaceutically acceptable solvates include hydrates.

The GSK-3 inhibitors referred to herein are conveniently prepared according to the methods disclosed in the above mentioned patent publications in which they are disclosed. Thus, a compound of formula (IA), and/or a derivative thereof, may be prepared using the processes described in WO 00/21927; a compound of formula (IB), and/or a derivative thereof, may be prepared using the processes described in WO 00/38675, a compound of formula (IB'), and/or a derivative thereof, may be prepared using the processes described in WO 00/38675, a compound of formula (IC), and/or a derivative thereof, may be prepared using the processes described in WO 01/09106 and a compound of formula (ID), and/or a derivative thereof, may be prepared using the processes described in WO 01/74771.

The present invention also provides a GSK-3 inhibitor or a pharmaceutically acceptable derivative thereof, for use in the promotion of nerve regeneration, for example axonal regrowth, axonal outgrowth, and the prevention of growth cone collapse, in cases of acute neuronal injury, such as crush injury, acute stroke, ischaemia, neurotraumatic insult, spinal cord injury and neurotrauma.

The present invention also provides a GSK-3 inhibitor or a pharmaceutically acceptable derivative thereof, for use in the promotion of nerve regeneration, for example axonal regrowth, axonal outgrowth, and the prevention of growth cone collapse, in cases of chronic neurodegenerative conditions such as Alzheimer's disease, Parkinson's disease, neurodegenerative diseases, Lou Gehrig's disease, and multiple sclerosis.

The present invention also provides a GSK-3 inhibitor or a pharmaceutically acceptable derivative thereof, for use in the promotion of nerve regeneration, for example axonal regrowth, axonal outgrowth, and the prevention of growth cone collapse, in cases of Parkinson's Disease, Lou Gehrig's disease and multiple sclerosis.

In a preferred aspect, said GSK-3 inhibitor is a compound described in WO 00/21927, WO 00/38675, WO 01/09106 and WO 01/74771 or a pharmaceutically acceptable derivative thereof.

The present invention also provides a GSK-3 inhibitor or a pharmaceutically acceptable derivative thereof, for use in the manufacture of a medicament for the promotion of nerve regeneration, for example axonal regrowth, axonal outgrowth, and the prevention of growth cone collapse, in cases of acute neuronal injury, such as crush injury, acute stroke, ischaemia, neurotraumatic insult, spinal cord injury and neurotrauma.

The present invention also provides a GSK-3 inhibitor or a pharmaceutically acceptable derivative thereof, for use in the manufacture of a medicament for the promotion of nerve regeneration, for example axonal regrowth, axonal outgrowth, and the prevention of growth cone collapse, in cases of chronic neurodegenerative conditions such as Alzheimer's disease, Parkinson's disease, neurodegenerative diseases, Lou Gehrig's disease, and multiple sclerosis.

The present invention also provides a GSK-3 inhibitor or a pharmaceutically acceptable derivative thereof, for use in manufacture of a medicament for the promotion



of nerve regeneration, for example axonal regrowth, axonal outgrowth, and the prevention of growth cone collapse, in cases of Parkinson's Disease, Lou Gehrig's disease and multiple sclerosis.

5 In a preferred aspect, said GSK-3 inhibitor is a compound described in WO 00/21927, WO 00/38675, WO 01/09106 and WO 01/74771 or a pharmaceutically acceptable derivative thereof.

In the above mentioned methods the GSK-3 inhibitor, may be administered per se or, preferably, as a pharmaceutical composition also comprising a pharmaceutically acceptable carrier.

10 In the treatment of the invention, the GSK-3 inhibitor mentioned herein is formulated and administered in accordance with the methods disclosed in the above mentioned patent applications and patents.

Accordingly, the present invention also provides a pharmaceutical composition for the promotion of nerve regeneration, for example axonal regrowth, axonal outgrowth,  
15 and the prevention of growth cone collapse, in cases of acute neuronal injury, such as crush injury, acute stroke, ischaemia, neurotraumatic insult, spinal cord injury and neurotrauma, which composition comprises a GSK-3 inhibitor, or a pharmaceutically acceptable derivative thereof, and a pharmaceutically acceptable carrier therefor.

Accordingly, the present invention also provides a pharmaceutical composition for the promotion of nerve regeneration, for example axonal regrowth, axonal outgrowth,  
20 and the prevention of growth cone collapse, in cases of chronic neurodegenerative conditions such as Alzheimer's disease, Parkinson's disease, neurodegenerative diseases, Lou Gehrig's disease, and multiple sclerosis, which composition comprises a GSK-3 inhibitor, or a pharmaceutically acceptable derivative thereof, and a pharmaceutically  
25 acceptable carrier therefor.

Accordingly, the present invention also provides a pharmaceutical composition for the promotion of nerve regeneration, for example axonal regrowth, axonal outgrowth, and the prevention of growth cone collapse, in cases of Parkinson's Disease, Lou Gehrig's disease and multiple sclerosis, which composition comprises a GSK-3 inhibitor or a  
30 pharmaceutically acceptable derivative thereof, and a pharmaceutically acceptable carrier therefor.

In a preferred aspect, said GSK-3 inhibitor is a compound described in WO 00/21927, WO 00/38675, WO 01/09106 and WO 01/74771 or a pharmaceutically acceptable derivative thereof.

35 As used herein the term 'pharmaceutically acceptable' embraces compounds, compositions and ingredients for both human and veterinary use: for example the term 'pharmaceutically acceptable salt' embraces a veterinarily acceptable salt.

The composition may, if desired, be in the form of a pack accompanied by written or printed instructions for use.

40 Usually the pharmaceutical compositions of the present invention will be adapted for oral administration, although compositions for administration by other routes, such as by injection and percutaneous absorption are also envisaged.

Particularly suitable compositions for oral administration are unit dosage forms such as tablets and capsules. Other fixed unit dosage forms, such as powders presented in sachets, may also be used.

5 In accordance with conventional pharmaceutical practice the carrier may comprise a diluent, filler, disintegrant, wetting agent, lubricant, colourant, flavourant or other conventional adjuvant.

Typical carriers include, for example, microcrystalline cellulose, starch, sodium starch glycolate, polyvinylpyrrolidone, polyvinylpolypyrrolidone, magnesium stearate, sodium lauryl sulphate or sucrose.

10 Suitable dosages of the GSK-3 inhibitor include the known doses for these compounds as described or referred to in reference texts such as the British and US Pharmacopoeias, Remington's Pharmaceutical Sciences (Mack Publishing Co.), Martindale The Extra Pharmacopoeia (London, The Pharmaceutical Press) (for example see the 31st Edition page 341 and pages cited therein) or the above mentioned  
15 publications or doses which can be determined by standard procedures.

Suitable dosages of the compound of formula (IA) include those disclosed in WO 00/21927.

Suitable dosages of the compound of formula (IB) include those disclosed in WO 00/38675.

20 Suitable dosages of the compound of formula (IB') include those disclosed in WO 00/38675.

Suitable dosages of the compound of formula (IC) include those disclosed in WO 01/09106.

25 Suitable dosages of the compound of formula (ID) include those disclosed in WO 01/74771.

The composition of the invention may be administered from 1 to 6 times a day, but most preferably 1 or 2 times per day.

30 The solid oral compositions may be prepared by conventional methods of blending, filling or tableting. Repeated blending operations may be used to distribute the active agent throughout those compositions employing large quantities of fillers. Such operations are of course conventional in the art. The tablets may be coated according to methods well known in normal pharmaceutical practice, in particular with an enteric coating.

35 Oral liquid preparations may be in the form of, for example, emulsions, syrups, or elixirs, or may be presented as a dry product for reconstitution with water or other suitable vehicle before use. Such liquid preparations may contain conventional additives such as suspending agents, for example sorbitol, syrup, methyl cellulose, gelatin, hydroxyethylcellulose, carboxymethylcellulose, aluminium stearate gel, hydrogenated edible fats; emulsifying agents, for example lecithin, sorbitan monooleate, or acacia;  
40 non-aqueous vehicles (which may include edible oils), for example almond oil, fractionated coconut oil, oily esters such as esters of glycerine, propylene glycol, or ethyl alcohol; preservatives, for example methyl or propyl p-hydroxybenzoate or sorbic acid; and if desired conventional flavouring or colouring agents.

For parenteral administration, fluid unit dosage forms are prepared utilizing the compound and a sterile vehicle, and, depending on the concentration used, can be either suspended or dissolved in the vehicle. In preparing solutions the compound can be dissolved in water for injection and filter sterilized before filling into a suitable vial or ampoule and sealing. Advantageously, adjuvants such as a local anaesthetic, a preservative and buffering agents can be dissolved in the vehicle. To enhance the stability, the composition can be frozen after filling into the vial and the water removed under vacuum. Parenteral suspensions are prepared in substantially the same manner, except that the compound is suspended in the vehicle instead of being dissolved, and sterilization cannot be accomplished by filtration. The compound can be sterilized by exposure to ethylene oxide before suspending in the sterile vehicle. Advantageously, a surfactant or wetting agent is included in the composition to facilitate uniform distribution of the compound.

Compositions may contain from 0.1% to 99% by weight, preferably from 10-60% by weight, of the active material, depending upon the method of administration.

Compositions may, if desired, be in the form of a pack accompanied by written or printed instructions for use.

The compositions are formulated according to conventional methods, such as those disclosed in standard reference texts, for example the British and US Pharmacopoeias, Remington's Pharmaceutical Sciences (Mack Publishing Co.), Martindale The Extra Pharmacopoeia (London, The Pharmaceutical Press) and Harry's Cosmeticology (Leonard Hill Books).

The contents of the publications mentioned herein, with particular reference to the disclosures of International Patent Applications Publication Numbers WO 00/21927, WO 00/38675, WO 01/09106 and WO 01/74771 and the specific Examples mentioned therein, shall be considered incorporated herein by reference.

No adverse toxicological effects are expected for the compositions or methods of the invention in the above mentioned dosage ranges.

The following Examples illustrate the present invention but are not intended to limit it in any way.

### **Example 1**

#### **Biological Protocol**

##### **(a) Antibodies**

Mouse monoclonal anti-GSK-3 (clone 4G-1E) and anti-P-(Y279/216)-GSK-3 (clone 5G-2F) antibodies were obtained from UBI. Rabbit polyclonal antibody against P-(Ser9)-GSK-3 $\beta$  was purchased from Biosource. Mouse monoclonal GSK-3 $\beta$  antibody was obtained from Transduction Labs. Polyclonal sheep antibodies recognizing GSK-3 $\alpha$  and P-(Ser21)-GSK-3 $\alpha$  were purchased from UBI. Mouse monoclonal neurofilament

antibodies recognising the 68 kDa, 160 kDa, and the 200 kDa forms were obtained from Zymed and Sigma, and the monoclonal anti- $\beta$ -tubulin antibody was obtained from Sigma.

#### (b) Dorsal Root Ganglion Explant Culture and Collapse Assays

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Fertile eggs were obtained from a local supplier (Needle farm). DRG explants cultures from E7 chick embryos were prepared as previously described (Eickholt et al., *Mol. Cell. Neurosci.*, 1997, 9, 358). For immunohistochemistry DRG explants were plated onto poly-L-lysine (20 $\mu$ g/ml)/laminin (20 $\mu$ g/ml) coated glass coverslips, and cultures were incubated for 20 hours in DMEM/10% FCS/PenStrep, supplemented with 20ng/ml NGF and 1% penicillin/streptomycin before fixation in 4% PFA/10% sucrose. For collapse assays, DRGs were cultured for 24 hours on 20 $\mu$ g/ml laminin coated Labtec chamberslides (Nunc). LiCl (Sigma) and specific GSK-3 inhibitors ("SB-216763" and "SB-415286") were applied at given concentrations and incubated for 1 hour before the

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Sema 3A-Fc was applied (at 1 $\mu$ g/ml). After 30 minutes, the cultures were carefully fixed in 4% paraformaldehyde/10% sucrose.

#### (c) Neurite Outgrowth on Immobilized Semaphorin3A-Fc

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Glass coverslips were coated with 20 $\mu$ g/ml poly-L-Lysine and washed 3 times with PBS. A mixture of anti-Fc antibody (2 $\mu$ g/ml, Sigma) and laminin 1 $\mu$ g/ml was applied and incubated for 1 hour. After 3 washes with DMEM/10%FCS, Sema 3A-Fc supernatant (where stated) was applied and incubated for 1 hour. After several washes with DMEM/10% FCS isolated E7 DRGs were placed on the coverslips and cultured for

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20 hours in DMEM/10% FCS in the presence of 20ng/ml NGF.

#### (d) Immunocytochemical and Histochemical Procedures

PFA fixed DRG explants were washed twice with PBS, permeabilized in PBS/1% Triton and blocked in blocking buffer (PBS/0.5% triton/2% BSA). Primary antibody was applied (all antibodies were diluted 1:50 in blocking buffer, except anti-P(Y<sup>279</sup>/Y<sup>216</sup>)-GSK-3 antibody that was diluted 1:100) and incubated overnight at 4°C with agitation. Bound antibody was visualized using FITC conjugated secondary antibodies (Sigma, anti-sheep antibody was from DAKO). The distribution of filamentous actin was visualized using Texas-Red conjugated Phalloidin (Molecular Probes). All samples were

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analyzed using volume deconvolution. For paraffin wax sectioning, paraformaldehyde fixed chick embryos were embedded in wax, cut into 6  $\mu$ m sections, and processed according to previously described methods (Bancroft and Stevens, *Theory and practice of histological techniques*, Churchill Livingstone, London, 1996).

#### (e) Cell/tissue Lysates and Western Blot Analysis

Cos-7 cells were transfected using Lipofectamine plus reagents after the manufacturer's protocol. Transfected cells were washed with icecold PBS and lysed in lysis buffer (20mM Hepes, 150mM NaCl, 1% Triton, 5mM CaCl<sub>2</sub>, 1mM MgCl<sub>2</sub>, protease inhibitors). Brain lysate was prepared from E9 chick brains using same lysis conditions. After removing insoluble material by centrifugation, protein extracts were separated by SDS-PAGE (10%) and transferred onto nitrocellulose. Bound proteins were detected by

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western blotting. All primary antibodies were used at 1:1000. Secondary antibodies were purchased from Vector and used at 1:5000.

## **Results**

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Inhibition of GSK-3 Prevents Semaphorin 3A Induced Growth Cone Collapse and Overrides the Inhibitory Influence of Immobilized Semaphorin 3A (see Figure 1).

## 10 **Example 2**

### **Biological Protocol**

15 A similar protocol was undertaken to that described in Example 1 above. However, in this instance the effects of two GSK-3 inhibitors, SB-216763 and SB-331371 on growth cone collapse by Sema 3A were investigated.

## **Results**

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See Figure 2.

## **Discussion**

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With reference to Figure 1, the results of the biological protocol may be summarised as follows:

30 (a) **GSK-3 inhibitor-induced block of growth cone collapse by Sema 3A.**  
Addition of Sema 3A-Fc to DRG explant cultures for 30 minutes induces a growth collapse response. The graphs show % collapsed growth cone<sub>±sem</sub> (n≥4 independent experiments). In each experiment ≥100 growth cones were counted. In the presence of 20mM LiCl the Sema 3A response is substantially inhibited, whilst NaCl at 20mM did not alter the Sema 3A induced growth cone collapse. Likewise, the two specific GSK-3  
35 inhibitors, SB216763 and SB415286 (used at μM concentrations as stated), inhibited the Sema 3A induces growth cone collapse in a dose dependant manner.

40 (b) Examples of Phalloidin-stained DRG growth cones in order to visualize the distribution of the filamentous actin. First micrograph shows a control untreated (-) growth cone. All subsequent pictures show growth cones that have been treated with Sema 3A (+) in the absence (control) and presence of LiCl (20mM), SB216763 (10μM), and SB415286 (30μM). In the presence of the GSK-3 antagonist growth cone collapse is clearly inhibited. Scale bar, 15μm.

45 (c) **Immobilised Sema 3A is capable of inhibiting neurite outgrowth, an effect overcome by inhibition of GSK-3.**

DRG explants were cultured in the absence and presence of SB415286 for 20 hours on substrates consisting of pure laminin or on a mixture of laminin and affinity captured Sema 3A-Fc (see biological protocol). For the evaluation of the influence of

Sema 3A on neurite elongation the length of the 20 longest neurites that extended from isolated DRG explants cultured under the given culture conditions were measured ( $n \geq 5$ ).

(d) In the first micrograph DRG explant shows profuse axonal growth when cultured on a permissive laminin substrate. On a substrate consisting of a mixture of laminin and affinity captured Sema 3A-Fc, DRG axons remain in close proximity to the explant (middle). Following inhibition of GSK-3 with SB415286 at 30 $\mu$ M, neurons acquire an ability to extend onto the inhibitory laminin/Sema 3A substrate. Fluorescence micrographs show explants stained with an anti- $\beta$ -tubulin antibody. Scale bar, 250 $\mu$ m.

The results obtained from Example 2 were found to be consistent with those detailed above for Example 1.

### Summary

The results obtained from Examples 1 and 2 are consistent with the following findings –

(a) Inhibition of GSK-3 prevents the growth cone collapse response induced by Semaphorin 3A; and

(b) Inhibition of GSK-3 leads to stimulation of axonal regeneration into otherwise inhibitory Semaphorin 3A territory.

### Claims

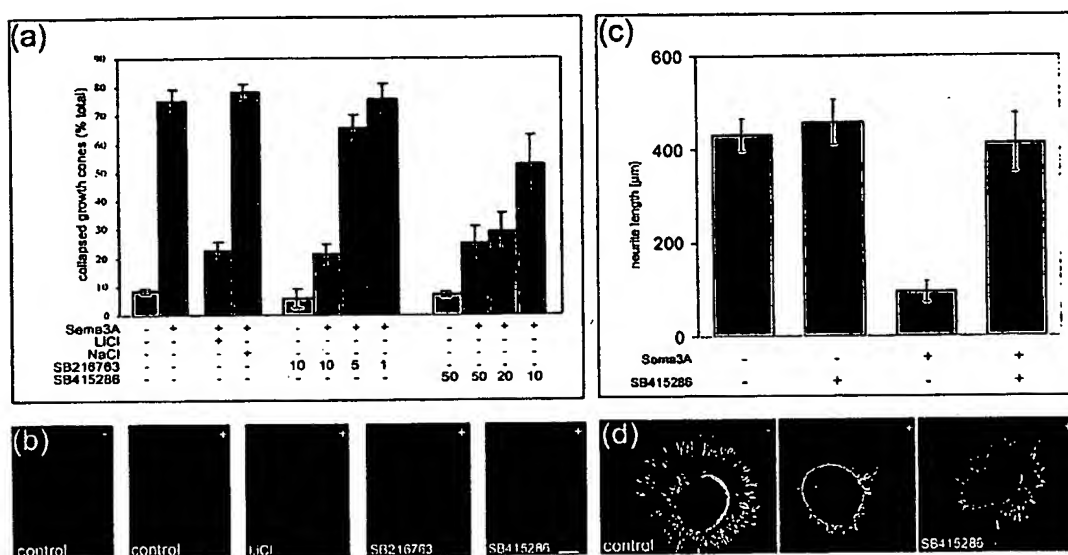
1. A method of treatment for the promotion of nerve regeneration, including axonal regrowth, axonal outgrowth, and prevention of growth cone collapse, in cases of acute neuronal injury, such as crush injury, acute stroke, ischaemia, neurotraumatic insult, spinal cord injury and neurotrauma in humans or non-human mammals, which method comprises the administration of an effective, non-toxic and pharmaceutically acceptable amount of a GSK-3 inhibitor or a pharmaceutically acceptable derivative thereof.
2. A method of treatment for the promotion of nerve regeneration, for example axonal regrowth, axonal outgrowth, and the prevention of growth cone collapse, in cases of chronic neurodegenerative conditions such as Alzheimer's disease, Parkinson's disease, neurodegenerative diseases, Lou Gehrig's disease, and multiple sclerosis, in humans or non-human mammals, which method comprises the administration of an effective, non-toxic and pharmaceutically acceptable amount of a GSK-3 inhibitor or a pharmaceutically acceptable derivative thereof.
3. A method of treatment according to claim 1 or claim 2, wherein said GSK-3 inhibitor, is selected from the compounds described in WO 00/21927, WO 00/38675, WO 01/09106 and WO 01/74771.
4. A GSK-3 inhibitor or a pharmaceutically acceptable derivative thereof, for use in the promotion of nerve regeneration, for example axonal regrowth, axonal outgrowth, and the prevention of growth cone collapse, in cases of acute neuronal injury, such as crush injury, acute stroke, ischaemia, neurotraumatic insult, spinal cord injury and neurotrauma.
5. A GSK-3 inhibitor or a pharmaceutically acceptable derivative thereof, for use in the promotion of nerve regeneration, for example axonal regrowth, axonal outgrowth, and the prevention of growth cone collapse, in cases of chronic neurodegenerative conditions such as Alzheimer's disease, Parkinson's disease, neurodegenerative diseases, Lou Gehrig's disease, and multiple sclerosis.
6. Use according to claim 4 or claim 5, wherein said GSK-3 inhibitor, is selected from the compounds described in WO 00/21927, WO 00/38675, WO 01/09106 and WO 01/74771.
7. A GSK-3 inhibitor or a pharmaceutically acceptable derivative thereof, for use in the manufacture of a medicament for the promotion of nerve regeneration, for example axonal regrowth, axonal outgrowth, and the prevention of growth cone collapse, in cases of acute neuronal injury, such as crush injury, acute stroke, ischaemia, neurotraumatic insult, spinal cord injury and neurotrauma.

8. A GSK-3 inhibitor or a pharmaceutically acceptable derivative thereof, for use in the manufacture of a medicament for the promotion of nerve regeneration, for example axonal regrowth, axonal outgrowth, and the prevention of growth cone collapse, in cases of chronic neurodegenerative conditions such as Alzheimer's disease, Parkinson's disease, neurodegenerative diseases, Lou Gehrig's disease, and multiple sclerosis.
9. Use according to claim 7 or claim 8 wherein said GSK-3 inhibitor, is selected from the compounds described in WO 00/21927, WO 00/38675, WO 01/09106 and WO 01/74771.
10. A pharmaceutical composition for the promotion of nerve regeneration, for example axonal regrowth, axonal outgrowth, and the prevention of growth cone collapse, in cases of acute neuronal injury, such as crush injury, acute stroke, ischaemia, neurotraumatic insult, spinal cord injury and neurotrauma, which composition comprises a GSK-3 inhibitor, or a pharmaceutically acceptable derivative thereof, and a pharmaceutically acceptable carrier therefor.
11. A pharmaceutical composition for the promotion of nerve regeneration, for example axonal regrowth, axonal outgrowth, and the prevention of growth cone collapse, in cases of chronic neurodegenerative conditions such as Alzheimer's disease, Parkinson's disease, neurodegenerative diseases, Lou Gehrig's disease, and multiple sclerosis, which composition comprises a GSK-3 inhibitor, or a pharmaceutically acceptable derivative thereof, and a pharmaceutically acceptable carrier therefor.
12. A pharmaceutical composition according to claim 10 or claim 11, wherein said GSK-3 inhibitor, is selected from the compounds described in WO 00/21927, WO 00/38675, WO 01/09106 and WO 01/74771.



**Figure 1**

**5     Inhibition of GSK-3 Prevents Semaphorin 3A Induced Growth Cone Collapse and**  
**Overrides the Inhibitory Influence of Immobilized Semaphorin 3A**



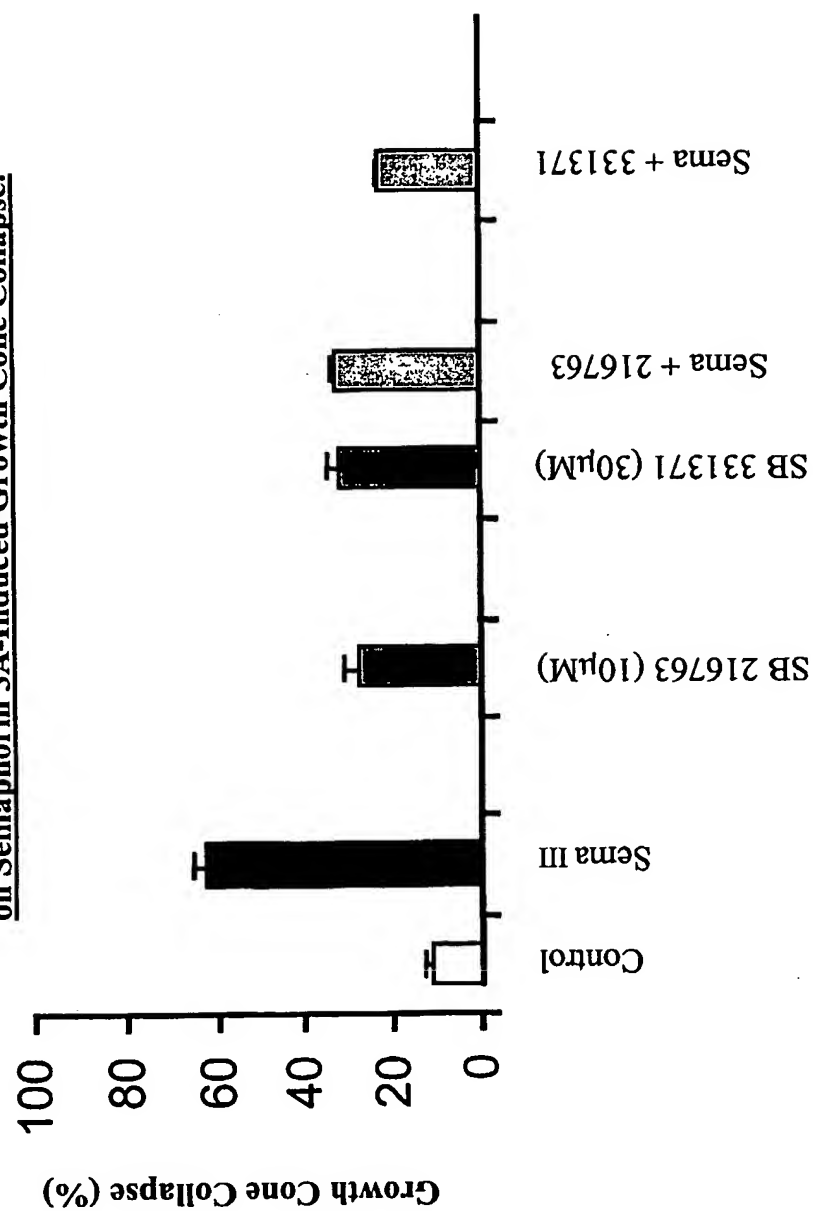
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**Figure 2**  
**Effects of Inhibitors of GSK-3**  
**on Semaphorin 3A-Induced Growth Cone Collapse.**



# INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 02/00542

**A. CLASSIFICATION OF SUBJECT MATTER**  
IPC 7 A61K45/00

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)  
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 01 74771 A (SMITH DAVID GLYNN ;WARD ROBERT WILLIAM (GB); HAIGH DAVID (GB); SLI) 11 October 2001 (2001-10-11) cited in the application claims 1,4,5	1-12
X	WO 00 38675 A (HOLDER JULIE CAROLINE ;SMITH DAVID GLYNN (GB); COGHLAN MATTHEW PAU) 6 July 2000 (2000-07-06) cited in the application claims 1,10,11	1-12
X	WO 00 21927 A (FENWICK ASHLEY EDWARD ;HOLDER JULIE CAROLINE (GB); SMITH DAVID GLY) 20 April 2000 (2000-04-20) cited in the application claims 1,22,23	1-12
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☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

**\* Special categories of cited documents:**

\*A\* document defining the general state of the art which is not considered to be of particular relevance

\*E\* earlier document but published on or after the international filing date

\*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

\*O\* document referring to an oral disclosure, use, exhibition or other means

\*P\* document published prior to the international filing date but later than the priority date claimed

\*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

\*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

\*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

\*Z\* document member of the same patent family

Date of the actual completion of the international search

15 May 2002

Date of mailing of the international search report

28/05/2002

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+31-70) 340-3016

Authorized officer

Beranová, P

## FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 1 - 12

Present claims 1 - 12 relate to an extremely large number of possible compounds. In fact, the claims contain so many options, variables, possible permutations and provisos that a lack of clarity and conciseness within the meaning of Article 84 EPC arises to such an extent as to render a meaningful search of the claims impossible. Consequently, the search has been carried out for those parts of the application which do appear to be clear and concise, namely the compounds described in WO 01/74771, WO 00/3867, WO 00/21927 and WO 01/09106.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 02/00542

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	<p>WO 01 09106 A (SMITH DAVID GLYNN ;WARD            ROBERT WILLIAM (GB); SMITHKLINE BEECHAM            PL) 8 February 2001 (2001-02-08)            cited in the application            claims 11-13</p> <p>-----</p>	1-12

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 02/00542

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 0174771	A	11-10-2001	AU 6215301 A WO 0174771 A1	15-10-2001 11-10-2001
WO 0038675	A	06-07-2000	AU 1877700 A EP 1140070 A1 WO 0038675 A1	31-07-2000 10-10-2001 06-07-2000
WO 0021927	A	20-04-2000	AU 6111699 A EP 1119548 A1 WO 0021927 A2	01-05-2000 01-08-2001 20-04-2000
WO 0109106	A	08-02-2001	AU 6989800 A WO 0109106 A1 EP 1200415 A1	19-02-2001 08-02-2001 02-05-2002